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Genetic Toxicity of Malathion: A Review

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Mammalian in vivo and in vitro studies of technical or commercial grade malathion and its metabolite malaoxon show a pattern of induction of chromosome damage, as measured by chromosome aberrations, sister chromatid exchanges, and micronuclei. Experiments with purified (>99%) malathion gave weak or negative results. In contrast to the cytogenetic effects of technical grade malathion, responses in gene mutation assays were generally negative except for malaoxon, which was positive for mammalian gene mutations in both tested instances. This result also could be a consequence of chromosome level changes, however. Dermal exposure, a common human route, caused cytogenetic damage in test animals at doses near those producing positive results by intraperitoneal injection. Workers who apply technical grade malathion and other pesticides have higher levels of chromosomal damage than unexposed individuals. Because of the inactivity of malathion mixtures in gene mutation assays, malathion has been thought to be of little genotoxic concern. However, the pattern of chromosome damage in animals and mammalian cells in culture (including human) indicates that technical grade malathion and its components have not been adequately studied for genotoxic potential in humans. © 1993 Wiley-Liss, Inc.

Key words: **chromosome damage, sister-chromatid exchange, human and animal in vivo and in vitro studies, organophosphate pesticides, human genotoxicity, malathion CAS 121-75-5, malaoxon CAS 1634-78-2**

INTRODUCTION

Malathion (CAS No. 121-75-5) (S-(1,2-dicarboethoxyethyl) O,O-dimethyldithiophosphate) is a broad spectrum insecticide introduced in 1950 by the American Cyanamid Company. For pest eradication a "commercial" or technical grade product is used which varies from approximately 90–95% malathion by weight and may contain approximately a dozen impurities formed during manufacture or storage. Among the important impurities is malaoxon, also the active metabolite. Malaoxon is formed from malathion by oxidation. Following malathion oxidation to malaoxon, the principal pharmacological activity is its inhibition of acetylcholinesterase enzymes in plasma, red blood cells, brain, muscle, and other tissues in mammals. Malathion is much more toxic to insects than to mammals due to the lower levels in insects of esterases which readily hydrolyse malathion and malaoxon to inactive products in mammals.

Malathion is widely used because of its relatively low acute toxicity compared to other organophosphorus insecticides. It has been employed in major eradication programs against insect infestations in metropolitan areas of Florida, Texas, and California [DHS, 1991]. The aerial application of malathion over large urban areas in southern California as part of the 1990 Mediterranean (Medfly) Eradication Program raised concerns over the potential for malathion to cause genetic damage. This was reviewed as part of the comprehensive health risk assessment of aerial application of malathion bait performed by the California Department of Health Services [DHS, 1991].

This review is organized to reflect the types of studies used to evaluate the genetic toxicity of malathion: animal in vivo studies; human and animal in vitro studies; gene mutation tests; human in vivo studies; and studies of DNA reactivity and mechanism. Plant studies were not included. Emphasis is placed on animal in vivo and human and animal in vitro studies, which provide the most pertinent evidence for purposes of risk assessment.

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ANIMAL IN VIVO STUDIES; CHROMOSOME ABERRATIONS AND MICRONUCLEI

Summary

Evidence of the genotoxic abilities of technical grade malathion is provided by results from several in vivo studies in which dermal, intraperitoneal (i.p.), or oral administration of technical grade malathion to test animals (mice or hamsters) produced statistically significant increases in chromosome damage to cells located in the bone marrow. Summary results of seven in vivo studies which measured chromosomal aberrations and micronuclei in mice or hamsters following exposure to technical grade or purified malathion are shown in Table I. Five studies with technical grade or other than pure malathion gave positive results for chromosome damage. Two studies with reportedly purified malathion were negative. Increased frequencies of chromosome aberrations [Salvadori et al., 1988; Dulout et al., 1983; Dzonkowska and Hubner, 1986] or micronuclei [Dulout et al., 1982] were observed in bone marrow cells at daily dose levels in the range of 120 to 250 mg/kg, and these effective dose levels decreased with repeated exposures. In a fifth study, positive results for chromosome damage were observed at a much lower daily dose of 1 mg/kg [Hoda and Sinha, 1991].

Methodology

The general methodology used in these studies is similar. Groups of 5 to 10 animals are given malathion via dermal or oral administration or i.p. injection. Single or repeated doses are administered in corn oil or water. Control groups receive only the vehicle solvent. The animals are sacrificed 6 to 36 hours after the final treatment and metaphase chromosomes from bone marrow or sperm cells examined for chromosome aberrations and micronuclei by standard cytogenetic methods. In some studies, colchicine was administered several hours prior to sacrifice to arrest cells in metaphase. The frequencies of chromosome gaps, breaks, exchanges, fragments, other structural abnormalities, or micronuclei in treated animals are compared with those in the controls. When the frequencies of chromosome aberrations or micronuclei in treated animals are significantly higher than those of the controls at or below the $P < 0.05$ level of significance, the results are judged to be positive.

Results and Discussion

Salvadori et al. [1988] reported chromosome damage in bone marrow and sperm cells of Swiss Webster mice. Dermal application of "commercial-grade" malathion (percent malathion not given) in corn oil to male mice resulted in two- to three-fold higher frequencies of chromosome aberrations (excluding "gaps") in bone marrow cells of treated animals as compared to controls. A positive-dose response relationship for chromosome aberrations was not observed. However malathion induced a significant dose-dependent decrease of mitotic indices in bone marrow cells. The lowest effective dose (LED) producing chromosome aberrations in bone marrow cells following a single application of malathion was 500 mg/kg bw. The LED following multiple treatments (five days per week for two weeks) was reduced to 250 mg/kg bw. The two-fold decrease in LED following multiple daily treatments suggests that the putative genotoxic compound(s) may accumulate over the ten-day multiple exposure period.

Salvadori et al. [1988] also examined chromosome aberrations in primary spermatocytes. The LED producing a statistically significant increase in chromosome aberrations (excluding gaps), following multiple treatment, was 500 mg/kg bw. Commercial grade malathion treatment also produced an increase in univalent chromosomes (those lacking centromeres). After multiple applications at a daily dose equal to or greater than 250 mg/kg bw, the frequency of univalents in spermatocytes from dosed animals was statistically significantly higher than the frequency in control animals. However, the positive findings in spermatocytes are suspect for a number of reasons. While higher frequencies of spermatocytes containing univalents were observed in both sex chromosomes and autosomes in malathion-exposed animals, the statistical strength of the effect was stronger in the sex chromosomes, diminishing the significance of this effect. Also, the reported increase in univalents among the sex chromosomes exhibited a positive dose-response relationship, whereas the increase among the autosomes did not. Thus, the results of this study indicate that malathion or its metabolites produce cytogenetic effects in bone marrow cells. The evidence for positive effects in testicular cells is questionable and warrants further investigation.

Dulout and co-workers observed statistically significant increases in micronuclei in bone-marrow cells of Rockland (Swiss) male mice [Dulout et al., 1982] and chromosome aberrations in bone marrow cells of BALB/c mice [Dulout et al., 1983] following either dermal administration or i.p. injection of 95.5% pure malathion in corn oil. These effects were measured following single administration of doses equal to or greater than 120 mg/kg bw (for micronuclei) or 230 mg/kg bw (for chromosome aberrations).

A weak positive result was obtained by Dzonkowska and Hubner [1986] who observed higher frequencies of chromosome aberrations (excluding "gaps") as compared to controls in bone marrow cells of female Syrian golden hamsters following a single i.p. injection of a water suspension containing 30% malathion at doses equal to or greater than 240 mg/kg bw. No dose-response was observed, perhaps because of cell-cycle delay. Among the aberrations observed were dicentric and ring chromosomes. The reported presence of such unlikely (perhaps artifactual) structural forms raises concerns about the cytogenetic analysis performed in this study. Dicentric and ring chromosome formation are

TABLE I. In Vivo Studies: Summary of Studies of Chromosome Aberrations and Micronuclei Following Exposure to Malathion*

Reference	Malathion purity	Species (strain)	Cell type	Route	Dosing regimen	LED (NOAEL) mg/kg	Result	Responses	
								Control	LED
A. Chromosome aberrations									
Studies with impure malathion									
Salvadori et al. [1988]	Commercial	Mouse (Swiss Webster)	Bone marrow	Dermal	5d/wk; 2wk	250	+	0.4^a	1.3
			Spermatocytes		5d/wk; 2wk	500	+?	0.7^a	1.7
Dulout et al. [1983]	95.5%	Mouse (BALB/c)	Bone marrow	i.p.	Single	230	+	0.9^a	2.2
Dzonkowska & Hubner [1986]	30%	Hamster (Syrian)	Bone marrow	i.p.	Single	240	+	0.0^a	1.2
Hoda & Sinha [1991]	50%	Mouse (Albino Swiss)	Bone marrow	Oral (intubation)	10d	1	+	1.3^a	7.3
Studies with purified malathion									
Degraeve et al. [1984]	>99%	Mouse (Q Strain)	Bone marrow	Oral (water)	5d/wk; 7wk	(1)	–	0.2^a	0.4
Degraeve & Moutschen	>99%	Mouse (Q Strain)	Bone marrow	i.p.	Single	(300)	–	0.4^a	0.6
B. Micronuclei									
Dulout et al. [1982]	95.5%	Mouse (Rockland Swiss)	Bone marrow	Dermal	Single	120	+	4.9^b	17.1
				i.p.	Single	120	+	4.8^b	8.5

*LED = Lowest Effective Dose, NOAEL = No-Observed-Adverse-Effect-Level, i.p. = intraperitoneal injection

[a] percent cells with chromosome aberrations

[b] percent cells with micronuclei

events typically associated with exposures to radiomimetic agents such as x-rays and agents which generate free radicals. In contrast, malathion is believed to be a weak alkylating agent (see below). Furthermore, even strong alkylating agents such as cyclophosphamide cause these types of events rarely, at frequencies only a few percent of those of more common aberrations such as chromosome and chromatid breaks. However, in this study, technical grade malathion reportedly induced dicentrics and ring chromosomes at one-third the frequency of breaks. The observation of an unexpectedly high frequency of these rare events relative to the frequency of breaks adds more uncertainty to the cytogenetic analysis.

Hoda and Sinha [1991] observed a five-fold increase in the incidence of total chromosome and chromatid abnormalities in bone marrow cells of malathion-fed inbred albino Swiss mice. Increased frequencies of chromatid breaks, fragments, multiple breaks, and chromosome loss were reported. A 50% pure pesticide formulation was administered orally by intubation, at a daily dose of approximately 1 mg/kg bw for ten days. The dose used in this study was more than two orders of magnitude lower than the lowest doses which gave positive responses in the four other in vivo animal studies above. No explanation can be provided for the very large difference in the lowest effect dose in this study (1 mg/kg bw) as compared with doses used in the other positive studies (120–240 mg/kg bw). However, it is noteworthy that the control responses in the mice used by Hoda and Sinha [1991] were higher than those in other positive studies; these inbred albino Swiss mice could have an increased sensitivity to damage.

Negative results for chromosome aberrations were reported in two studies by Degraeve and coworkers. Degraeve and Moutschen [1984] failed to observe an increase in aberrations in either bone marrow or sperm cells of mice (Q strain) given single i.p. injections of malathion (reported as >99% pure) at a dose of 300 mg/kg bw. Degraeve et al. [1984] also observed no increase in aberrations in bone marrow cells following oral administration of >99% pure malathion in drinking water to mice (Q strain) at a dose of 1 mg/kg bw, a dose more than 100 times lower than the doses used in four of the five above studies which gave positive responses. While these two studies reported use of >99% pure malathion, the description given in Degraeve and Moutschen of a "yellow-brown liquid" fits the description of technical grade, not purified, malathion. Thus, there is uncertainty about the grade actually used by these investigators.

It may be significant that the five studies which gave positive results employed relatively impure malathion (30–95.5%) while the one negative study conducted at a dose (300 mg/kg bw) comparable to those used in all but one of the positive studies (120–240 mg/kg bw) employed malathion which was reportedly >99% pure. This suggests that the presence of impurities in commercial or technical grade malathion may contribute to the observed cytogenetic effects. Although the evidence suggests impurities may play a role in technical grade malathion-induced cytogenetic effects, it should be emphasized that this is the grade of compound humans are exposed to and should be concerned about. Also, since malaoxon is one of the major impurities, the possibility of this compound having an effect should be considered.

HUMAN AND ANIMAL IN VITRO STUDIES

Summary

The chromosomal effects produced by technical grade or commercial malathion in test animals are generally supported by results from in vitro studies in cultured cells. Statistically significant increases in chromosome aberrations or sister chromatid exchanges (SCEs) are produced in human, hamster, or buffalo cell lines with or without metabolic activation. A summary of the results of in vitro cytogenetic studies of malathion and malaoxon is presented in Table II.

Increases in chromosomal aberrations in human lymphocytes or hamster ovary cells are reported in six studies with either technical grade and other than pure malathion [Herath et al., 1989; Ishidate et al., 1981; Gupta et al., 1988; Galloway et al., 1987; Garry et al., 1990] or purified malathion [Walter et al., 1980]. The studies of Galloway et al. [1987], Gupta et al. [1988] and Garry et al. [1990] provide adequate evidence of chromosome damaging effects in vitro. Positive effects reported in two other studies [Herath et al., 1989; Walter et al., 1980] are weak or equivocal, while in another [Ishidate et al., 1981] only summary data are presented.

All studies which examined the effects of technical grade or purified malathion or malaoxon on SCE frequencies in human and hamster cell lines are positive [Ivett et al., 1989; Herath et al., 1989; Galloway et al., 1987; Sobti et al., 1982; Chen et al., 1981, 1982; Nishio and Uyeki, 1981; Nicholas et al., 1979; Garry et al., 1990].

Methodology

The studies were carried out in cultures of freshly isolated blood cells from human donors or established animal or human cell lines. The cells are incubated in growth media in suspension or monolayer cultures. Malathion or malaoxon (50–99% pure) is dissolved in DMSO, ethanol, or water and administered to cells for treatment periods ranging from 4–96 hours. Control cultures receive only the solvents. In some studies, rat liver extracts are added to the cultures to provide additional metabolic activation. When the frequencies of cells with chromosome aberrations or SCEs in treated cultures are significantly higher than those of the untreated control cultures at or below the $P < 0.05$ level of significance, the results are judged to be positive.

TABLE II. Cell Culture: Summary of Studies of Chromosome Aberrations and Sister Chromatid Exchanges Following Exposure to Malathion and Malaoxon*

Reference	Malathion purity	Species	Cell type	Solvent	LEC (NOAEC) µg/mL		Result	Responses Control	LEC
A. Chromosome aberrations									
1. Malathion									
Studies with impure malathion									
Herath et al. [1989]	<98%	Human	Lymphocytes	DMSO	20	−S9	+	0.3^a	0.9
Ishidate et al. [1981]	unk.	Hamster	Lung	DMSO	76	±S9	+	unk.	unk.
Galloway et al. [1987)	94%	Hamster	CHO-WBL	DMSO	(76)	−S9	−	2.0^a	3.0
					303	+S9	+	0.0^a	10.0
Gupta et al. [1988]	50%	Buffalo	Lymphocytes	Water	25	−S9	+	4.0^a	7.5
Huang et al. [1973)	95%	Human	Human cell line	DMSO	(100)	−S9	+	2.0^a	5.0
Garry et al. [1990]	98%	Human	Lymphocytes	Surfactant	132	−S9	+	0.0^a	15.0
				(Pluronic F127)	83	+S9	+	2.0^a	10.0
Studies with purified malathion									
Walter et al. [1980]	99%	Human	Lymphocytes	EtOH	10	−S9	+	0.1^a	0.6
2. Malaoxon									
Ivett et al. [1989)	94%	Hamster	CHO-WBL	DMSO	(377)	−S9	−	1.0^a	2.0
					3010	+S9	+/−?	1.0^a	18.0
B. Sister chromatid exchanges									
1. Malathion									
Studies with impure malathion									
Herath et al. [1989]	<98%	Human	Lymphocytes	DMSO	20	−S9	+	4.8^b	7.4
Garry et al. [1990]	98%	Human	Lymphocytes	Surfactant	650	−S9	+	4^b	6
				(pluronic F127)	650	+S9	+	4.5^b	7
Sobti et al. [1982]	98%	Human	B cell	EtOH	20	−S9	+	7.0^b	14.1
					20	−S9	+	6.0^b	11.0
					20	−S9	+	5.9^b	10.9
Galloway et al. [1987]	94%	Hamster	CHO-WBL	DMSO	40	−S9	+	10.5^b	18.3
					300	+S9	+		
Chen et al. [1981]	94%	Hamster	V79 fibroblasts	DMSO	40	+S9	+	5.9^b	7.6
Chen et al. [1982]	94%	Hamster	V79 fibroblasts	DMSO	40	−S9	+	4.2^b	7.4
Studies with purified malathion									
Nicholas et al. [1979]	99%	Human	Fibroblasts	EtOH	20	−S9	+	8.6^b	12.0
					20	−S9	+	9.4^b	14.7
Nishio and Uyeki [1981]	99%	Hamster	CHO	DMSO	100	−S9	+	9.4^b	14.2
2. Malaoxon									
Nishio and Uyeki [1981]	96%	Hamster	CHO	DMSO	3	−S9	+	9.4^b	14.2
Ivett et al. [1989]	94%	Hamster	CHO-WBL	DMSO	124	−S9	+	8.6^b	15.0

[a] Percent cells with chromosome aberrations

[b] Sister chromatid exchanges per cell

*LEC = Lowest Effective Concentration, CHO = Chinese hamster ovary (cells), NOAEC = No-observed-adverse-effect-concentration, DMSO = Dimethylsulfoxide, EtOH = Ethanol, S9 = Metabolic activation system to metabolize malathion

+ = Positive response

− = Negative response

RESULTS

Chromosome Aberrations

A. Malathion

Among the six malathion studies reporting positive effects for chromosome aberrations, the clearest positive result was obtained by Garry et al. [1990], who exposed whole human blood to malathion (98% pure) emulsified in a surfactant, Pluronic F127, with and without S9 (a rat liver homogenate). Concentration-related increases in chromosomal aberrations (both simple and complex) relative to solvent controls were obtained. The differences between treated and untreated cultures were statistically significant at nontoxic malathion concentrations. The lowest effective concentrations with and without S9 were 83 μg/mL and 132 μg/mL, respectively. (The lowest concentrations tested with and without S9 were 33 μg/mL and 66 μg/mL, respectively). Complex structural aberrations (dicentric and tetraradial forms) were most frequently observed in the presence of S9. This study provides evidence of chromosomal damaging activity in vitro by relatively pure (98%) malathion.

The study of Galloway et al. [1987] also provides unequivocal evidence for chromosome aberrations, but only at cytotoxic concentrations. Treatment of cultured Chinese hamster ovary (CHO) cells with malathion (94% pure) in dimethylsulfoxide produced statistically significant increases in chromosome aberrations (both simple and complex), but only at very high concentrations which produced cell cycle delay. The lowest effective concentration (LEC) giving positive effects was 303 μg/mL, and results were positive only with the addition of an exogenous metabolic activation system. The LEC of 303 μg/mL is approximately twice the maximum solubility of malathion in water, and at these concentrations of malathion a precipitate in the cultures was observed.

Gupta et al. [1988] treated buffalo lymphocytes with malathion (50% purity in water) and observed higher frequencies of chromosome aberrations among treated cells than among controls. Treatment produced a positive concentration-response relationship, and statistically significant increases were observed at malathion concentrations of 25 μg/mL and higher without addition of exogenous metabolic activation. The highest concentration (50 μg/mL) produced the highest response (22% cells with chromosome aberrations) which was more than four times the response in the control cultures (4% cells with chromosome aberrations).

Weak or equivocal positive results for chromosome aberrations were obtained by Herath et al. [1989] and Walter et al. [1980] in cultured human lymphocytes using malathion formulations of <98% or 99% purity, respectively. Neither study employed exogenous metabolic activation. The reports of both of these studies lack important technical details (e.g., incomplete presentation of methodologies, failure to indicate if gaps were included) and, therefore, offer limited evidence of the ability of malathion to induce chromosome aberrations.

Ishidate et al. [1981] reported that the application of malathion (of undefined purity) to cultures of Chinese hamster lung fibroblasts at concentrations up to those causing 50% growth inhibition produced greater percentages of cells with chromosome aberrations compared to solvent-treated controls. However, it is difficult to assess the merits of the study because methodology and results are reported only in summary form.

Huang [1973] observed no increase in chromosome aberrations (gaps plus breaks) in a cultured human hematopoietic cell line following administration of malathion (95%) at concentrations as high as 100 μg/mL. No exogenous metabolic activation was added.

B. Malaoxon

In the only study of malaoxon, Ivett et al. [1989] observed a positive response in CHO cells in one trial following treatment at very high toxic concentrations (3 mg/mL) of 94% pure test material; this positive result, however, was not repeatable in two other trials.

Sister Chromatid Exchange (Malathion and Malaoxon)

There is general agreement that both technical grade and pure malathion and impure malaoxon can induce SCEs in cultured human and animal cells, even without added metabolic activation, giving weak but statistically significant effects. The lowest effective concentration of malathion ranged from 20–100 μg/mL in seven positive studies [Galloway et al., 1987; Nishio and Uyeki, 1981; Herath et al., 1989; Sobti et al., 1982; Nicholas et al., 1979; Chen et al., 1981, 1982]. In an eighth positive study [Garry et al., 1990], a higher malathion concentration (650 μg/mL) was required, perhaps because fewer metaphases were scored. Garry et al. scored 20 metaphase cells per dose, whereas in the other positive studies 40–70 metaphases were scored, thus increasing sensitivity. In two studies of impure malaoxon, positive responses in CHO cells were observed in which the lowest positive response in CHO cells ranged from 33–124 μg/mL [Ivett et al., 1989; Nishio and Uyeki, 1981].

DISCUSSION

Chromosome Aberrations

The studies of Garry et al. [1990] and Galloway et al. [1987] provide adequate evidence that other than pure malathion damages chromosomes of cells in culture. Garry et al. [1990] obtained a clear concentration-dependent response both with and without metabolic activation; statistically significant responses were observed at concentrations near the limit of malathion's water solubility. The LECs with and without S9 were 83 μg/mL and 123 μg/mL, respectively. Garry et al. [1990] did not observe toxicity at these concentrations perhaps because cells were exposed to malathion emulsified in a surfactant. Galloway et al. [1987] obtained positive results in the presence of added metabolic activation but only at high toxic concentrations (LEC = 303 μg/mL), above the maximum solubility of malathion; tests were not conducted at non-toxic, soluble concentrations.

The Gupta et al. [1988] study also showed a positive response for chromosome damage. The effects were concentration-dependent, did not require metabolic activation, and occurred at much lower malathion concentrations (LEC = 25 μg/mL) than in the above two studies.

The differences in LECs and requirements for metabolic activation found in these three positive studies may stem from differences in the test materials and experimental systems employed. Garry et al. [1990] and Galloway et al. [1987] used malathion of 98% and 94% purity, respectively, while Gupta et al. [1988] used malathion of 50% purity. If cytogenetic effects are due to impurities, then the higher level of impurities in the material used by Gupta and coworkers could account for the much lower effective concentration observed in their study. Gupta also used cells from buffalos which has a higher reported background level than human cells, and so could have an increased sensitivity to damage.

Galloway et al. [1987] obtained positive results in CHO cells, but only with added metabolic activation. The differences in the requirements for metabolic activation between these studies may arise from differences in the experimental systems: buffalo and human lymphocytes may metabolize malathion to active form(s) more readily than CHO cells.

Sister Chromatid Exchange

Technical grade malathion and malaoxon cause an increase in SCE frequency in all studies, which indicates that these chemicals, or their impurities or metabolites, can affect DNA. The mechanism of genetic damage which results in sister chromatid exchanges is not well understood, but some correlation with induction of single strand breaks has been found [Thompson et al., 1990].

GENE MUTATION TESTS

Malathion (technical grade and purified) and malaoxon (94% pure) have been evaluated in a large number of gene mutation tests that employ bacteria (including the Ames *Salmonella* test and several sensitive *Escherichia coli* reversion assays), yeasts, insects, and animal cells in culture. The results of these gene mutation tests are summarized in Table III. In 15 studies using standard gene mutation assays, malathion was negative. In three studies where malathion of unknown purity was administered to bacterial cells in suspension, weak positive results were reported. Malaoxon was negative in the Ames test, but positive in two studies in mouse lymphoma cells using 94% pure test material at high concentrations. Since the mouse lymphoma (thymidine kinase) assay detects both chromosomal aberrations and point mutations [Moore and Doerr, 1990], the positive finding with malaoxon would support either a clastogenic mechanism or one involving point mutagenesis. A clastogenic mechanism is consistent with findings that malaoxon induces cytogenetic damage (e.g., SCEs) but a point mutation mechanism cannot be entirely ruled out.

Thus, malathion (technical grade or purified) does not appear to induce point mutation in DNA in bacterial systems. However, in the only two published mammalian cell studies, malaoxon (94% pure) was positive in a gene mutation assay which detects both chromosomal and point mutations.

HUMAN IN VIVO STUDIES

The evidence from human studies in the literature is insufficient to conclude whether technical or commercial grade malathion produces genetic damage in humans. Pesticide applicators with exposures to technical grade malathion and other insecticides have higher levels of chromosomal damage (aberrations) or SCEs than do populations without such exposures [Rupa et al., 1988, 1989, 1991; Paldy et al., 1987; Dulout et al., 1985; DeFerrari et al., 1991; Yoder et al., 1973; Crossen et al., 1978]. Exposures to other mutagenic or clastogenic factors (other pesticides, cigarette smoking, and alcohol consumption) were not adequately controlled for in many of these studies. In a study of patients acutely intoxicated with technical grade malathion, a temporary but significant increase was found in the frequency of chromosome aberrations [van Bao et al., 1974]. These studies are summarized in Table IV.

Results from epidemiological studies do not provide definitive evidence that malathion is genotoxic to humans. Although there are eight positive studies, exposures to other pesticides and additional confounders make the results with regard to malathion difficult to interpret. However, the chromosome aberrations associated with acute poisoning from technical grade malathion alone indicate that technical grade malathion is capable of causing temporary damage in humans at high exposure levels. The positive findings from animal in vivo and animal cell tests in vitro indicate that more adequate tests of genetic effects in humans are needed.

DNA REACTIVITY AND MECHANISM

Several studies have examined the reactivity of technical grade and purified malathion with DNA. Wiaderkiewicz et al. [1986] presented weak evidence that malathion (99% pure) can methylate DNA bases in vitro, suggesting that the

TABLE III. Gene Mutation Tests of Malathion and Malaoxon

Test system and reference	Result	Comment
A. Malathion		
Bacteria:		
Salmonella (Ames) reversion		
McCann et al. [1975)	Negative	
Shirasu et al. [1976)	Negative	
Wildeman and Nazar [1982]	Negative	
Waters et al. [1982]	Negative	
Shiau et al. [1980]	Negative	In quantitative assay; authors conclude pos. in TA1535 at 300 μg/plate in spot test
Haworth et al. [1983]	Negative	
Pednekar et al. [1987]	Negative	
Wong et al. [1989]	Negative	
Shigaeva and Savitskaya [1981]	Weak Positive	With preincubation, in TA1535-S9; LEC = 16.7 μg/mL
Ishidate et al. [1981]	Weak Positive	With preincubation, in TA100 +S9; summary data only, so independent evaluation difficult
Bacillus subtilis forward mutation		
Shiau et al. [1980]	Weak Positive	With preincubation, −S9 activation; LEC = 12.5 μg/mL
E. coli K-12 forward mutation		
Mohn (1973)	Negative	
E. coli WP2 reversion		
Ashwood-Smith et al. (1972)	Negative	
Shirasu et al. (1976)	Negative	
Waters et al. (1982)	Negative	
Moriya et al. (1983)	Negative	
Yeasts:		
S. pombe forward mutation		
Gilot-Delhalle et al. (1983)	Negative	
Insects:		
D. Melanogaster point mutation		
Velazquez et al. (1987)	Negative	
B. Malaoxon		
Bacteria:		
Salmonella (Ames) reversion		
Tennant et al. [1987]	Negative	
Zeiger et al. [1988]	Negative	
Mammalian cells in culture:		
Mouse lymphoma		
Tennant et al. [1987]	Positive	−S9 activation; LEC = 123.5 μg/mL
Myhr and Caspary (1991)	Positive	−S9 activation; LEC = 150 μg/mL

major methylation product is 7-methylguanine. A well conducted study by Imamura and Talcott [1985] demonstrated that malathion (99% pure) and several of its impurities alkylate nitrobenzylpyridine, a synthetic substrate. However, none of the impurities was mutagenic in the Ames *Salmonella* test. Malaoxon was not one of the impurities studied. Malathion (99% pure) caused slight denaturation of calf thymus DNA in solution [Olinski et al., 1980]. Griffin and Hill [1978] and Richardson and Imamura [1985] demonstrated malathion-induced breakage of bacterial plasmid or bacteriophage DNA in vitro; the purity of the test material was not reported. Houk and DeMarini [1987] found no evidence of *E. coli* phage induction by malathion (73.5% pure). A strong decrease in mitotic index was shown in other in vitro and in vivo studies using technical grade or other than pure malathion [Sobti et al., 1982; Rupa et al., 1991; Salvadori et al., 1988]. Malathion has therefore been shown to interact with DNA but the major mechanism of damage is essentially unknown. High concentrations of malathion (99% pure) inhibited DNA replication in cultures of human lymphocytes [Czajkowska and Walter, 1980]. Wiszkowska et al. [1986] and Walter and Wiszkowska [1990] showed decreases in RNA polymerase activity in porcine and calf thymus cell nuclei and porcine peripheral blood lympho-

TABLE IV. Epidemiological Studies: Summary of Chromosome Aberrations or Sister Chromatid Exchanges in Lymphocytes of Cohorts Exposed to Pesticides (or Alcohol)*

Reference	End point	Country	Job or exposure	Malathion exposure	Other exposures	Result	% CAbs (SCEs per cell)	
							Control	Exposed
A. Pesticides								
Rupa et al. [1989]	CAbs	India	Spray applicators	+	+Pest; −C; −Alc	+	0.7	4.6
Rupa et al. [1988]	CAbs	India	Garden	+	+Pest; +C; +Alc	+	1.6	7.6
	SCEs					+	(7.6)	(12.6)
Rupa et al. [1991]	SCEs	India	Mix/spray	+	+Pest; −C; −Alc	+	(3.6)	(8.5)
Paldy et al. [1987]	CAbs	Hungary	Mix/spray	?	+Pest; +C; +Alc	+	1.1	4.3
Dulout et al. [1985]	Dics	Argentina	Floriculture	?	+Pest; +C; +Alc	+?	0.1	0.4
	SCEs					+	(5.5)	(6.5)
van Bao et al. [1974]	CAbs	Hungary	Acute overdose	+	+C?; +Alc?	+	3.3	20.4[a] 4.2[b]
Yoder et al. [1973]	CAbs	USA	Spray	+	+Pest; +C; +Alc	+	0.3	1.6
Crossen et al. [1978]	SCEs	New Zealand	Spray	−	+Pest; +C; +Alc	+	(7.6)	(9.3)
DeFerrari et al. [1991]	CAbs	Italy	Floriculture	+	+Pest; −C; +Alc?	+	5.0	10.2
	SCEs				+Pest; −C; +Alc?	−	(3.9)	(5.1)
B. Alcohol								
Obe et al. [1980]	CAbs	Germany	Alc	−	+C?	+	3.2[c]	6.8[c]
			Alc		+C	+	2.4[d]	5.3[d]
Butler and Sanger [1981]	SCEs	USA	Alc	−	+C?	+	(8.4)	(10.6)

*CAbs = percent cells with chromosome aberrations, SCEs = sister chromatid exchanges, Dics = dicentric aberrations, C = cigarette smokers, Alc = alcohol drinkers, Pest = pesticides.

[a] Time of overdose.

[b] Six months later.

[c] Controls (1–4 yrs); exposed (>10 yrs).

[d] Controls (nonsmokers); exposed (smokers).

cytes using purified malathion. These published studies suggest a weak interaction of malathion (technical grade and purified) with DNA.

CONCLUSIONS

The available evidence indicates that technical grade or other than pure malathion has the potential to produce genotoxic effects in mammalian systems, including humans. The results of the available studies on the genotoxicity of malathion can be summarized as follows:

- In test animals, technical grade malathion appears to have the potential to produce chromosomal changes including chromosomal aberrations and micronuclei.
- In humans, the genotoxic effects of malathion have not been adequately studied.
- In human and animal cells in culture, both technical grade and purified malathion appear to produce cytogenetic damage, including chromosomal aberrations and SCEs.
- Malathion does not appear to produce point mutations in standard gene mutation assays in bacteria, but its metabolite malaoxon (94% pure) was positive in mammalian cell mutation tests.
- The interaction of malathion with DNA has not been adequately studied; published studies suggest a weak interaction.

The human health implications of these data are unclear. Agents such as malathion, which cause mutations in in vitro or in vivo test systems, are not now regulated by health agencies as human hazards [EPA, 1986]. They may, however, have the potential to cause cancer or adverse reproductive outcomes in humans. A large body of evidence links mutations with these health outcomes. Because mutations, especially those resulting in the loss of tumor suppressor genes, are implicated as important mechanisms of human cancers [Cavenee et al., 1986; Gallie and Worton, 1986; Weinberg, 1989], data on mutational mechanisms becomes relevant. Several recent articles discussed the important role of chromosomal damage in cancer, and the possible underlying mutational mechanisms, such as mitotic recombination [Ramel, 1988; James et al., 1989; Kovacs and Kung, 1991]. Among adverse reproductive outcomes, spontaneously aborted abnormal embryos and fetuses frequently carry chromosomal abnormalities [Boue et al., 1985]. Chromosomal aberrations have long been known to be associated with various types of mental retardation and morphological abnormalities in man. For example, chromosomal imbalance (e.g., trisomy) is a cause of Down's Syndrome [Smith and Berg, 1976] and congenital cardiac malformations [Rowe and Uchida, 1961]. In a recent study of mechanism, a chromosomal translocation which accompanies a form of muscular dystrophy was shown to cause a deletion mutation in a specific gene locus that in turn causes the aberrant phenotype [Love and Davies, 1989]. These observations which stress the importance of chromosomal damage may indicate that exposure to malathion (and perhaps other organophosphates) has the potential for genetic damage in humans. It should be emphasized that genotoxic effects observed with malathion may in fact arise from impurities in the technical grade mixtures.

Additional support for the significance of the findings about malathion reviewed in this manuscript is also provided by studies recently reviewed by Garrett et al. [1992] showing that other organophosphates like malathion have similar cytogenetic effects. Dimethoate, for example, has many similar effects as well as structural similarity.

In conclusion, although the genotoxic effects observed with malathion may arise from impurities, including malaoxon in the technical grade mixtures, it should be emphasized that humans are exposed to the technical grade. To resolve the ambiguities related to the major causative agent(s) of the genotoxicity observed in impure mixtures, it is recommended that the composition of the technical grade malathion be determined and the components be tested individually for genotoxicity. Finally, since dermal exposure is a primary means of exposure in humans, it is important to observe that positive results were obtained in animals dermally exposed to technical grade malathion.

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